301R IX71 User Guide

Ver. 2.6

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November 5, 2011

FOREWORD:

This 301R_IX71 User Guide provides a detail overview of the optical system in 301R ESS which includes Olympus IX71 inverted bright field and dark field microscopy, phase contrast microscopy, lasers, visible light spectrometer, TIRF and confocal system.

All the instruments inside 301R belong to professor Fan-Gang Tseng, Nano-/Micro-Bio-Opto-Electro-Mechanical Systems and Fluidics Lab, ESS, NTHU.

Please read the usage rules before using and obey it severely.

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A. 301R_Optical System Operating Rules

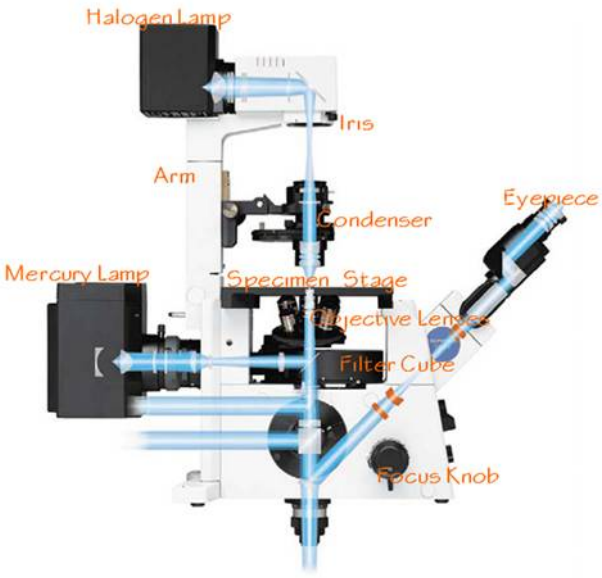
1. The equipment will only be available for qualified users from 10：00 to 22：00 weekdays.
2. Super-user is always available on weekdays and on the weekend. However, the reservation duration for super-users can't over 12 hours from 10:00 to 22:00 (Monday to Friday)
3. We strongly suggest qualified users book the time table in advance. However, every qualified user can directly use the equipment at following situations: (1) the time table has not been booked and no one is using. (2) Someone booked the time table in advance, but he/she is late to use it over 20 min.
4. General users can reserve less than 6 hours (including 6 hr) every week. Besides, we encourage general users to train new users, so general users can increase the quota, one hour per training time. When it achieved 12 hours, the user can be promoted to be a super-user after chief super-user's evaluation.
5. The person who wants to get the license of this system should follow the training rules and pass the qualification exam.
6. NO eating or drinking in the lab of optical system (Room 301). Please be caution of the liquid sample. If it's possible, please cover the machine connections to protect the equipment from broken by liquid solution. If, unfortunately, the liquid sample drops onto the machine connections or inside, please stop operating and contact the super-user immediately.
7. The user will get a personal DVD-RW or VCD-RW after qualifying exam. **Because the infection of the computer virus will result in the failure of some functions, to download the data should only use DVD or VCD. USB IS NOT allowed, or you will lose your license for a month. Furthermore, if the computer infected by virus due to your improper usage, we will cancel your license for two months, starting from the windows system of the computer can be used.**
8. If you had not used the system for over three months, we would have canceled your license

temporarily till one more qualifying exam.

9. The system contains three extra-sensitive CCDs that cost 4 million NT dollars. Moreover, they are easy to be broken due to improper usage and the fee of repair is quite expensive. Therefore, we ask every user to operate carefully. However, if the equipment is broken unfortunately due to your negligence or improper usage, we will report it to Prof. Tseng and suspend your license.

B. 301R_Optical System

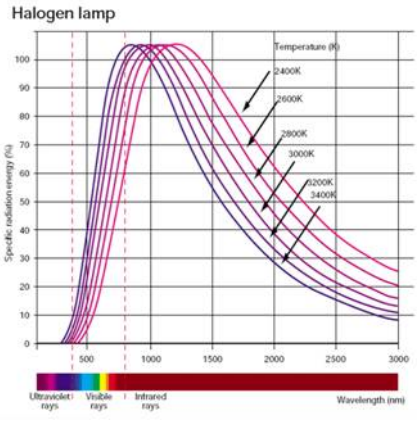
1. IX71 Inverted Microscopy

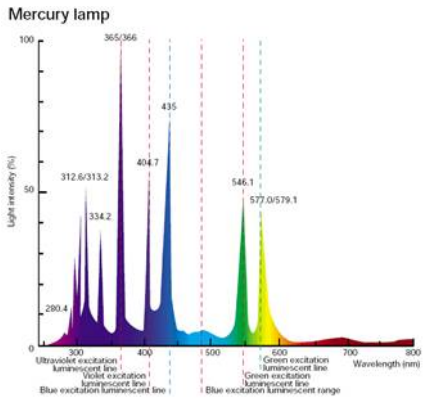


2. Light Sources

(1) Halogen Lamp: 20W (5~22 lm/W) (Spectrum as shown below/ left).

(2) Mercury Lamp: 100W (~50 lm/W) (Spectrum as shown below/ right).





(3) Lasers: 488 nm blue laser、532 nm green laser and 638 nm laser

3. Objective Lenses

Mag.	1x	2x	4x	10x
Color code	Black	Gray	Red	Yellow
Mag.	20x	40/ 50x	60x	100x
Color code	Green	Light blue	Dark blue	White

Function	Bright Field & Fluorescence	Dark Field	Phase Contrast	TIRF	Confocal System
Suited objectives	Anyone except the ones which are special used for TIRF and phase contrast	1. 60x NA.:0.65-1.25; WD.:120μm. *fig.1 2. 100x NA.:0.6-1.3; WD.: 200μm. *fig.2	1. 20x ph, N.A.:0.4; W.D.:3.2mm *fig.4 2. 60x ph, N.A.:0.7; W.D:1.5-2.2mm (adjustable) *fig.5	60x special use for TIRF NA.:1.4; W.D.:100μm *fig.6	1. 60x /NA.:0.65-1.25; WD.: 130μm. *fig.1 2. 100x /NA.:0.6-1.3; WD.:200μm. *fig.2 3. 100x /NA.:1.4; WD.:130μm. *fig.3
Note	Always turn the NA. to max.	Need a DF condenser, and always turn the NA. to min.	Need a phase contrast condenser	Objective marked "TIRF"	Always turn the NA. to max.

Fig.1

Fig.2

Fig.3

Fig.4

Fig.5

Fig.6

4. Filter Cube

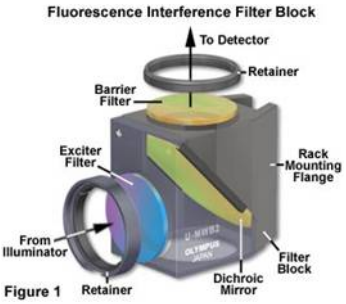


Figure 1

N0.1: 50/50 beam splitter

No.2: 505 dichroic mirror (DM)

No.3: Blank (No filter)

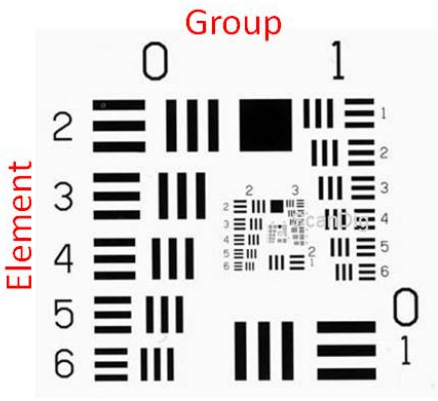
No.4: Ex. 470-490BP /505DM /Em. 510LP

No.5: Ex. 510-550BP /570DM /Em. 570-640BP

No.6: Ex. 590-650BP /660DM /Em. 667-738BP

5. CCD

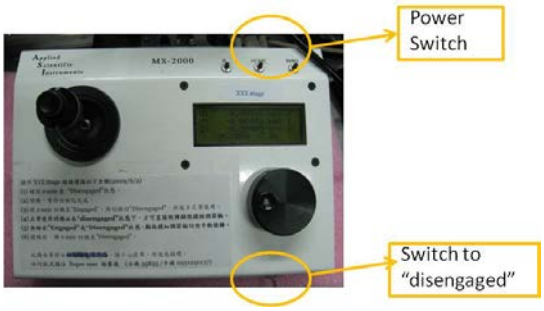
6. Scale Correction (Inside Dry Cabinet)



C. Based Usage of Microscopy (Bright Field or Fluorescence Microscopy)

1. Check the book of user record.
2. Turn on the Pc1.

3. Turn on the XYZ-stage-controller, and check that if the Z-position-controlled is switched to "disengaged". If it's not at the status of "disengaged", switch to "disengaged".



4. The X- or Y-axis of the sample is controlled by the XYZ-stage-controller. Only, the Z-position is controlled by the "focusing knobs" on the IX71 microscopy.

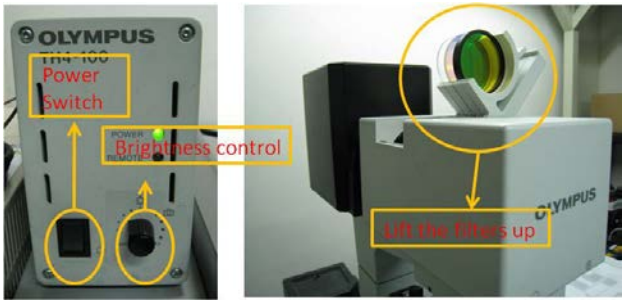
   NOTE: If there is noise from the knob or it moves automatically, please stop operating it and contact the super-user immediately.

5. Turn on the mercury lamp if it's essential for your experiment (the interval between turn on and off must be over 15 min.).

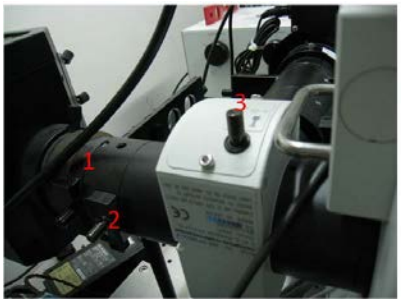
6. Turn the halogen lamp on, if it's needed. Increase the brightness for the observation and lift all the filters up on the part of the microscope.

   NOTE: When you want to switch the bright field or dark field (in which you use halogen lamp as the source) to fluorescence (in which you use mercury lamp as the source), **please turn off**

the halogen lamp first.



7. Take off the condenser lens of the dark field.
8. Lower down the stage for protecting it from collision between the objectives and the sample and then install an appropriate objective (Oil objective always be placed in the dry cabinet for storage).
9. Check the switches on the optical pathway.

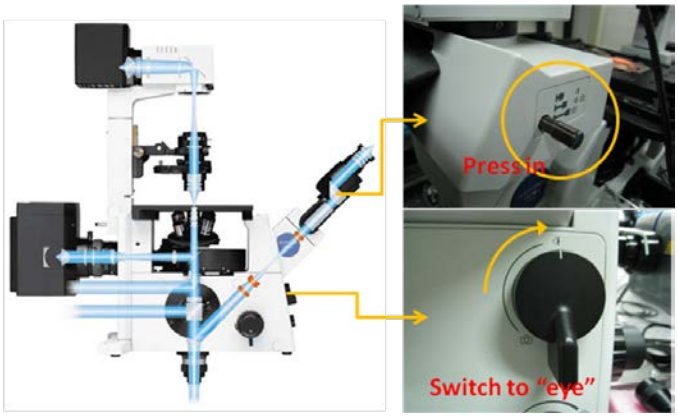


1. ND filter: brightness adjusted
2. Aperture: always full open
3. Pull up to let the light from mercury lamp can pass

10. Choose the filter cube (No.1→ 50/50 BS, No.2→DR505 FRET, No.3→bypass (no filter), No.4→ 488nm ex., No.5→ 532nm ex., No.6→ 638nm ex.).

SEE ALSO: **B. 301_R Optical System – 4. Filter Cube**

11. Eyepiece switch: Upper one in the figure below: full press in the bar, lower one in the figure below: switch to the “eyei con”.



12. Place the sample (upside down, which means cover glass in the bottom) and find the focal plane by using the Z-axial focusing knob.
13. Use EM CCD (black/white) or Pixel-Fly color CCD to capture image or record videos. In this

function, there're two CCDs can be used. Choose the one which is suitable for your experiment.

SEE: **E. EM CCD** or **F. Pixel-Fly CCD**

14. **Download the data by DVD or VCD only. USB IS NOT allowed.**
15. Turn off the PC1 after data-download.
16. Switch off the XYZ-stage controller.
17. Turn off all the lamps.
18. Lower the stage and take off the objective lens.
19. Use the cleaning paper and hemostat to clean the oil objective lens with the MeOH or EtOH(as shown below), and then put the cleaned objectives into the dry cabinet.

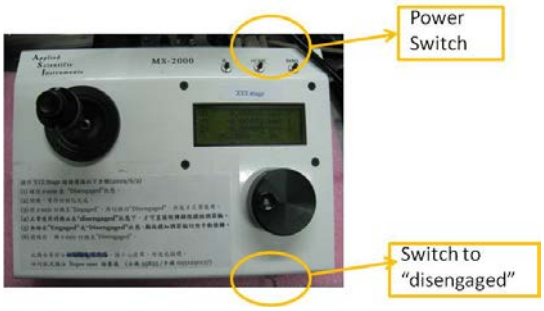
20. Close the filters.

21. Clean the table.
22. Write down the user record in the book.

D. Phase Contrast Microscopy and Dark Field Microscopy

1. Check the use record.
2. Turn on the PC1(the right one)

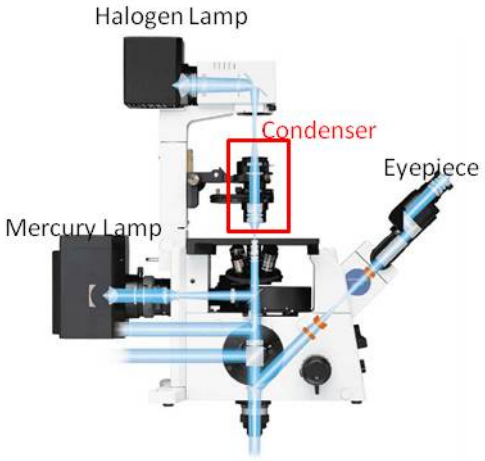
3. Turn on the XYZ-stage-controller, and check that if the Z-position-controlled is switched to "disengaged". If it is not at the status of "disengaged", switch to "disengaged".

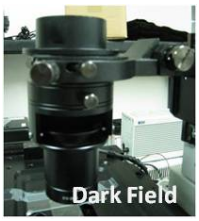


4. The X- or Y-axis of the sample is controlled by the XYZ-stage-controller. Only the Z-position is controlled by the "focusing knobs" on the IX71 microscope.

   NOTE: If there is noise from the knob or it moves automatically, please stop operating it and contact super-users immediately.

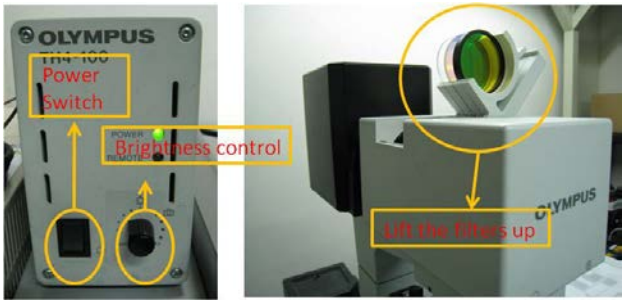
5. Choose the appropriate condenser dependent on what you need (phase contrast or dark field) and put it on the place as shown below.



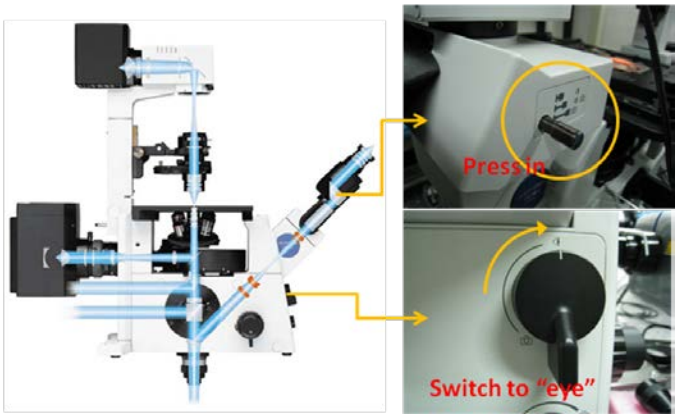


6. Turn the halogen lamp on, if it's needed. Increase the brightness for the observation and lift all

the filters up on the top part of the microscope.



7. Eyepiece switch: Upper one in the figures below: fully press in the bar; Lower one in the figures below: switch to the “eye icon”.

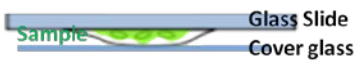


8. Lower down the stage for protecting it from collision between the objectives and the sample stage. Then install an appropriate objective. **(For phase contrast: 20x ph and 60x ph objective lens; For dark field: 100x oil and 60x oil objective lens, and all of them are placed in the dry cabinet).**

   SEE ALSO: **B. 301_R Optical System – 3. Objective lenses**

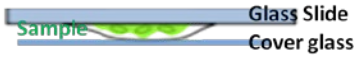
For Phase Contrast (Use 20x ph or 60x ph objective lens (with adjustable thickness of cover glass/W.D.),and all of them are placed in the dry cabinet)

9. Choose a matching annulus ring for the objective that you used.
10. If you use the 60x ph objective lens, adjust the thickness of cover glass to 0.17 mm, but the 20x ph one.
11. Choose the filter cube No.3 (bypass).
12. Place the sample (upside down, as shown below) and raise the objective lens holder slowly by using the Z-axial coarse focusing knob to find the focal plane. **Go to step 19.**



For Dark Field (Use 60x oil or 100x oil objective lens (both them can adjust the N.A. value), which placed in the dry cabinet)

13. Adjust the N.A. value of objective lens to the minimum (60x: 0.65; 100x: 0.65) and drop oil on the objective (about 1~2 drops).
14. Choose the filter cube No. 3(bypass)
15. Drop the oil on the objective lens (about 1-2 drops) and then place the sample (upside down, as shown below) and raise the objective lens holder slowly by using the Z-axial coarse focusing knob till the objective lens touch the sample.



16. Lower the condenser lens to ~5 mm from the sample.
17. Turn the Z-axis **fine** focusing knob to find the focal plane (if you can't get clear image, try to adjust the condenser up or down [±5 mm], and **avoid the collision between the condenser and the objective**)
18. If you want to switch to the fluorescence, please change filter cube to No.4-6 based on the fluorescent type. At the meantime, please adjust the N.A. value of the objective to maximum (60x: 1.25; 100x: 1.3).

NOTE: When you want to switch the bright field or dark field (in which you use halogen lamp as the source) to fluorescence (in which you use mercury lamp as the source), please **turn off the halogen lamp first**.

19. **Users frequently make a mistake that you don't switch cubes precisely. Then, it would be harmful to users' eyes or the CCDs. Therefore, please double check this step.**
    (Phase Contrast Mode: cube 3/the N.A. value are constant; DF Mode: cube 3/minimal N.A. value; Fluorescence Mode: cube 4-6/maximal N.A. value. Caution: NEVER use filter cube No.1~No.3 in Fluorescence mode, or it damages the CCDs or your eyes.)
20. Use EM CCD (black-white) or Pixel-Fly color CCD to capture images or record videos. In this function, there're two CCDs can be used. Choose the one which is suitable for your experiment.
    SEE: **E. EM CCD** or **F. Pixel-Fly CCD**
21. **Download the data by DVD or VCD only. USB IS NOT allowed.**
22. Turn off the PC1 after data-download

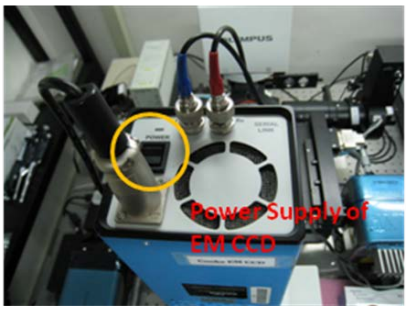
23. Switch off the XYZ-stage controller.
24. Turn off all lamps.
25. Take off the condenser and put it into the dry caninet.
26. Lower the stage and take off the objective lens.
27. Use the cleaning paper and hemostat to clean the oil objective lens with the MeOH or EtOH(as shown follows), and then put it into the dry cabinet.

28. Close the filters.

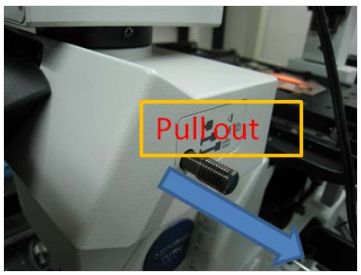
29. Clean the table.
30. Write the user record.

E. EM CCD (Electron-Multiplying Charge-Coupled Device, can take black/white image only, but fast frame rate.**)**

1. Install the EM CCD.
2. Turn on the power supply of extensions (as shown below, left figure, the third one from upside) and turn on the power of EM CCD (right figure).

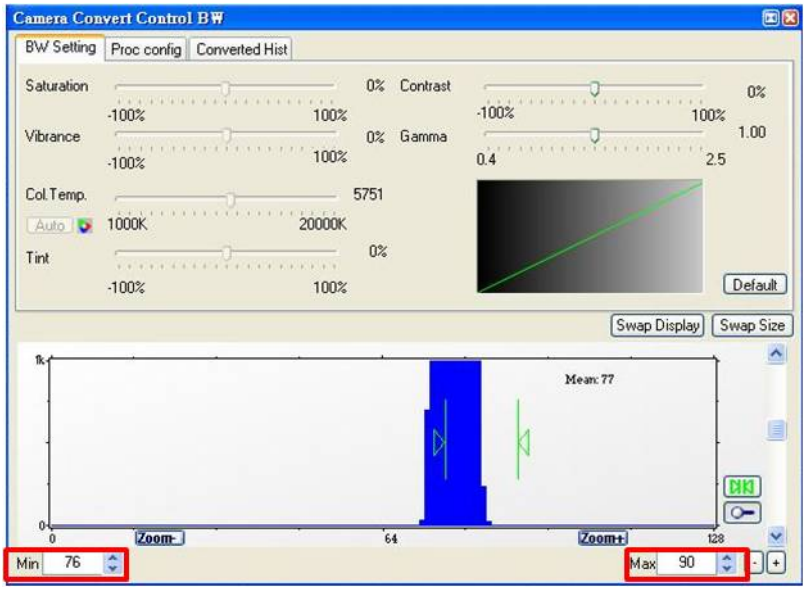


3. Click “CamWare” on the desktop, PC1.
4. Pull out the bar for photographing by EM CCD



5. Click for a new window of black/ white vision.
6. **Parameters setup,** click
   (1) Timing: Exposure time: 0.1 msec. (if the exposure less than 1 sec., please choose “short exposure time”, else, choose “long exposure time”). **The exposure time always begin 0.1 msec., and the increase can’t over 5 times of the last value.**
   (2) Analog mode: gain=2. **Gain value can increase as follows only: 2→5→10→20→50, the maximal value is 50.**
   (3) Binning: horiz. →1, vert.→1
   (4) Camera mode: video.
   (5) Trigger mode: intern.
7. Click for preview.
8. If the image is not clear enough, please click , and adjust the max. and min. to optimize

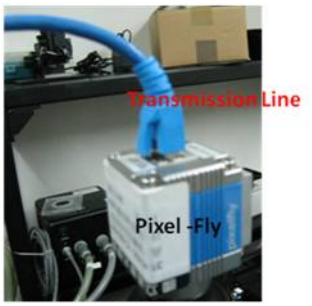
the contrast of image(as shown below, the red rectangles.), or click to auto-optimize.



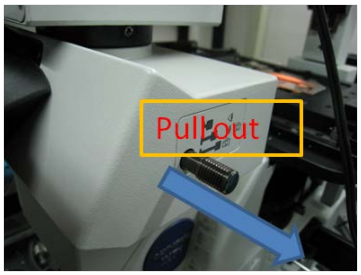
9. Click for single capturing images (click to rewind once)
10. File→export, export as 8-bit.tif (only one picture)
11. For video, click to start video record, to stop record, to play recording.
12. File→export record, can export as 8-bit.avi (video) or 8-bit.tif (pictures).
13. Save the data to your file.
14. If you won't use the mercury lamp any more, switch the power supply of mercury lamp off to prolong the it's life.
15. Reset the parameters of "CamWare"
    (1) Timing: Exposure time: 0.1 msec.
    (2) Analog mode: gain=2
16. Close the window→ switch off the power supply of EM CCD→switch the power supply of extensions.

F. Pixel-fly CCD (can take black/white or colorful image.**)**

1. Install the Pixel-Fly CCD.
2. Link up the transmission line with Pixel-fly CCD as shown below.

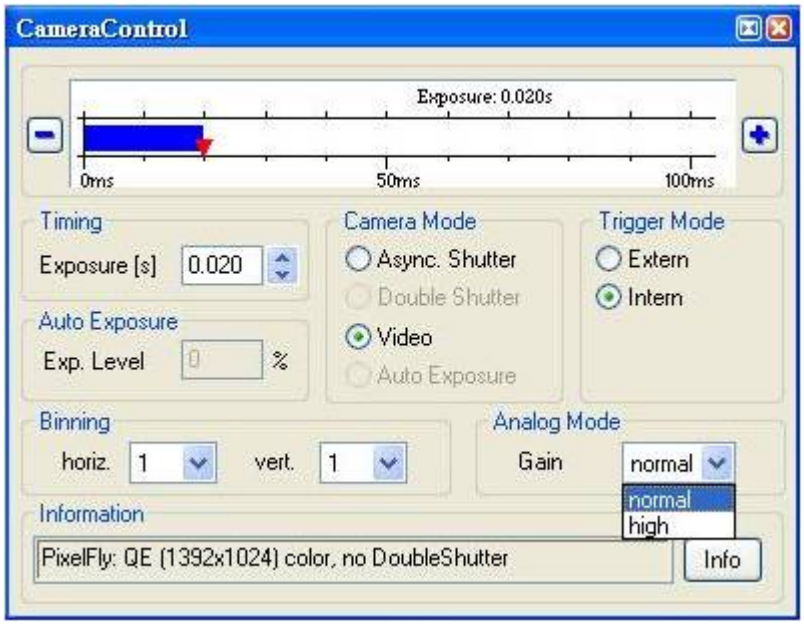


3. Click “CamWare” on the desktop, PC1.
4. Pull out the bar for photographing by Pixel-fly



5. Click for a new window of black/ white vision or click for a new window of color vision.

6. **Parameters setup**, click
   (1) Timing: Exposure time: 0.1 msec.. **The exposure time always begin 0.1 msec., and the increase can’t over 5 times of the last value.**
   (2) Analog mode: gain=normal. There are only two kinds of gain value, “normal” and “high”, of Pixel-fly CCD. If the contrast of your image is always not enough, try the high gain value.
   (3) Binning: horiz. →1, vert.→1
   (4) Camera mode: video.
   (5) Trigger mode: intern.

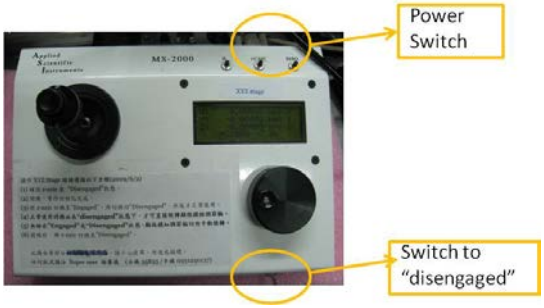


7. Click for preview.
8. Click for single capturing images (click to rewind once)
9. File→export, export as 8-bit.tif (only one picture).
10. For video, click to start video record, to stop record, to play recording.
11. File→export record, can export as 8-bit.avi (video) or 8-bit.tif (pictures).
12. Save the data to your file.
13. If you won't use the mercury lamp any more, switch the power supply of mercury lamp off to prolong the it's life.
14. Please use the DVD or VCD for data-download.
15. Reset the parameters of "CamWare"
    (1) Timing: Exposure time: 0.1 msec.
    (2) Analog mode: gain=normal
16. Close the window→take off the transmission line of Pixel-fly.

G. Spectrometer

1. Check the book of user record.
2. Turn on the PC1 and PC2.

3. Remote Desktop Control (RDC) the PC2 by the PC1 (click “NE301.RDP”on the desktop of PC1, P.W.:35807) (if it can’t be connected, please try it again after 5-10min).
4. Turn on the XYZ-stage-controller, and check that if the Z-position-controlled is switched to “disengaged”. If it’s not at the status of “disengaged”, switch to “disengaged”.



5. The X- or Y-axis of the sample is controlled by the XYZ-stage-controller. Only, the Z-position is controlled by the “focusing knobs” on the IX71 microscopy.

   NOTE: If there is noise from the knob or it moves automatically, please stop operating it and contact the super-user immediately.

6. Turn on the mercury lamp if it’s essential for your experiment (the interval between turn on and off must be over 15 min.).

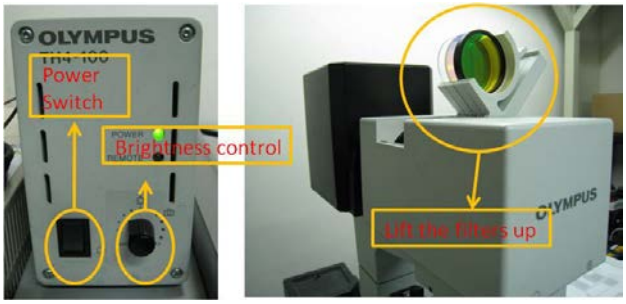
7. Switch on the power supply of extensions, and **then turn on the cooling system (left fig.), power source of spectrometer (middle fig.) and the CCD power source (right Fig.)**

sequentially.

8. Install the condenser if it's essential.

   SEE ALSO: **C. Phase Contrast and Dark Field Microscopy**

9. Turn on the halogen lamp on, if it's needed. Increasing the brightness for the observation and lift all the filters up on the part of microscopy.

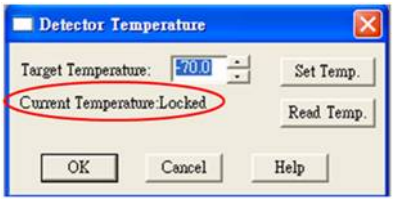


   NOTE: When you want to switch the bright field or dark field (in which you use halogen lamp as the source) to fluorescence (in which you use mercury lamp as the source), please turn off the halogen lamp first.

10. Lower down the stage for protecting it from collision between the objective and the sample and the operation is depending on the objective lens what you chose.
11. Choose the filter cube. (No.3→ bypass(no filter), No.4→488 nm ex. , No.5→532 nm ex. , No.6 638 nm ex.)

    SEE ALSO: **B. 301_R Optical System – 4. Filter Cube**

12. Turn the knob which below the eyepiece to the "eye icon"
13. Place the sample on the stage
14. Find the sample by using the specimen focusing knob.
15. Double click "Winspec" on the desktop, PC2
16. Setup → Detect Temp. .Close the window after the temperature down to -70 degree C (**DO NOT** do any operation before it achieved).



For Photograph

17. Spectrograph→Move→1200 Mirror. And key in “0 nm”.
18. Press down the bar, which is on the spectrometer, for photograph (pressing down the bar means the pinhole size is 1 cm^2 , in other says, the image size for the sample that we can observed is 1 cm^2)
19. Switch the knob to the “eye icon”, then take the background (Acquisition → Acquire Background)
20. Switch the knob to the “camera”.

21. **Parameters setup,**

    Acquisition→Experimental Setup, or quick button,

    (1) →ADC→Read out (choose “Normal”).

    (2) →Main, Gain value: 0 and Exposure time：0.1 msec.

    The exposure time always begin 0.1 msec., and the time-increasing can’t over 5 times of the last value every time.

    (3) Click “acquire” to take picture (as shown below), or quick button, .

NOTE: **The image NEVER be over-exposure, because this CCD has no protector. Please move the sample slowly especially when the sample has very different signal regionally**. And for the different range of wavelength-scanning, please reduce the exposure time to 0.1 msec. and then increase again slightly.

22. There is no the video mode, in other words, it can only take the picture.
23. Save image: File→save as, save as the 8-bit .tif.

For Spectrum (don't need to take the background anymore)

24. Move the sample by stage, which you're interested in, to the center of the image, and pull up the bar which is on the spectrometer (the range for the sample which can be observed is 1 mm^2 now)
25. Spectrograph→Move→ 600 BLZ. Then, key in the fluorescence peak wavelength of your sample (in the region of 400~700 nm).
26. **Parameters setup,**

    Acquisition→Experimental Setup or quick button,

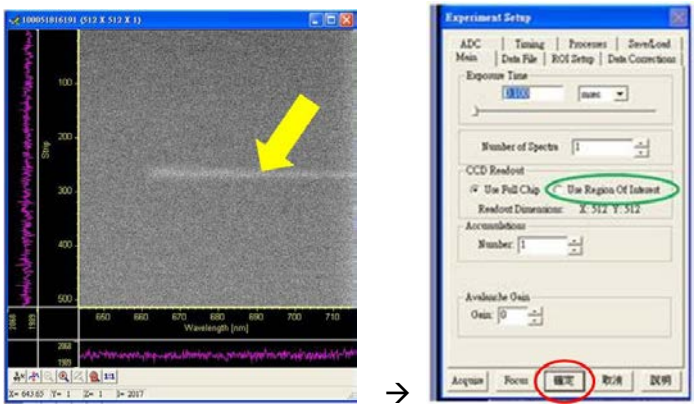
    (1) →ADC→Read out (choose "multiple gain").

    (2) →Main, Gain value: 0 and Exposure time：0.1 msec. The exposure time always begin 0.1 msec., and the time-increasing can't over 5 times of the last value. We can start the gain if it still can't take any obvious image while the exposure time already arrive in 1 sec. (start value: 500→1000, and then increase step by step (+500), maximal value: 3000).

    (3) Click "acquire" to take spectrum (as shown below), or quick button, .

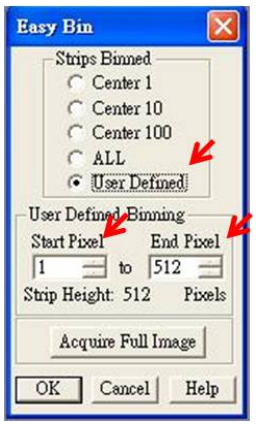
    (4) After click "acquire"→ click the right button of the mouse→New...→Graph

    (5) When there appear a bright line on the center (as shown below/ left), click acquisition→ choose "Use Region of Interest" (as shown below/ right) → "確定".



(6) Acquisition→Easy Bin...

(7) Click “User Defined ”, and then adjust “Start Pixel” and “End Pixel” to circle the bright line with red square→OK.



(8) Click “acquire” to take spectrum again, or quick button, .

(9) The spectrum may be inverse when we choose “multiple gain”, for transforming the spectrum into normally, please click setup→hardware→Display→Reverse, and take the spectrum again.

HITS:

If the intensity counts are always over 60,000, which always due to the overheating, even then reduced the exposure time and gain value to minimum (0.1 msec. and 0). Please try

(1) Check the pipes of cooling system, and make sure if the water keeps flowing, else, adjust the pipes or check if the junctions (as shown below) have loosened.

(2) If it still don’t work, please try to reset the software:

① Setup→Hardware→Controller/Camera→Launch Camera Detection Wizard→確定→下一步(N)→下一步(N)→下一步(N)→完成.

② Setup→Hardware→Cleans→Load Default Values→是(Y)→確定→close window.

③ Try again from step 24.

NOTE: **The image NEVER be over-exposure, because this CCD has no protector. Please move the sample slowly especially when the sample has very different signal regionally**. For the different range of wavelength-scanning, please reduce the exposure time to 0.1 msec. and then increase again but slight. Same as the different wave length scanning)

HITS:

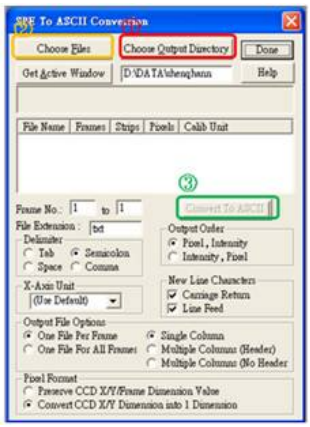
It could be a worked data when the single arrived 2,000~3,000 counts.

For the same sample, when the exposure time is lesser than 1 sec.(0.1 msec.~ 1sec.), increasing the exposure time slowly(5 times) without added gain value.

When the exposure time is between 1 sec. to 1 min., increasing the exposure time slightly (twice or three times) with a constant gain value (3000), however, when the exposure time is larger than 1 min. and the gain value is also arrived 3000 counts, and it still have no the enough data counts for the experiment, please asked super-users' help.

27. Export as the ".txt": Tool→convert to ACSII

(1) →Choose output directory and choose your folder for saving data →choose files and choose the data that you want to transfer



NOTE: The software will auto-save as "SPE Files (*.SPE*)" in C:/program files/PI acton/WinSpec

(2) Click “convert to ACSII”.

(3) After it show “done”, close the window.

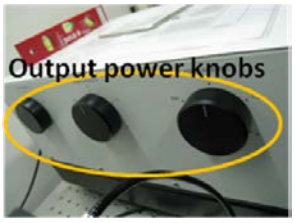
28. Turn off the Mercury lamp if you don’t need it any more in the latter experiment(s).
29. Please the DVD or VCD for data-download. **USB IS NOT allowed.**
30. Reset the parameters of the “Winspec”.
    (1) Exposure time: 0.1 msec.
    (2) gain value: 0.
    (3) ADC→Read out → Normal
    (4) Close the “Winspec”.
31. Switch the knob to “eye icon”.
32. Turn off the CCD power supply→spectrometer supply → cooling system supply→extend line supply(s). (never mistake the order for protect the CCD and computer from crash)

33. After burns the record, turn off the PC2 (move the mouse to the toolbar and click the right button of mouse→工作管理員(K)→關機(U)→關機(U)→確定)
34. Turn off PC1
35. Switch off XYZ stage controller
36. Switch off all lamps
37. Lower down the stage and take off the objective lens
38. Clean the oil objective lens with the MeOH or EtOH, and then put it into the dry cabinet.
39. Close the filters.

40. Clean the table.
41. Write the user record.

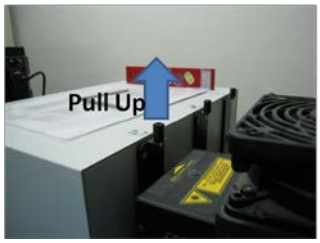
H. Laser (488 nm/~7 mW、532 nm/~30 mW、638 nm/~11 mW)

1. Check the use record. The time interval between switch on and off of every laser must be over 20 min.
2. Close all the shutters on the combiner (left figure).
3. Turn the knobs to make the output power be 0 (right figure), and switch on the laser you need.

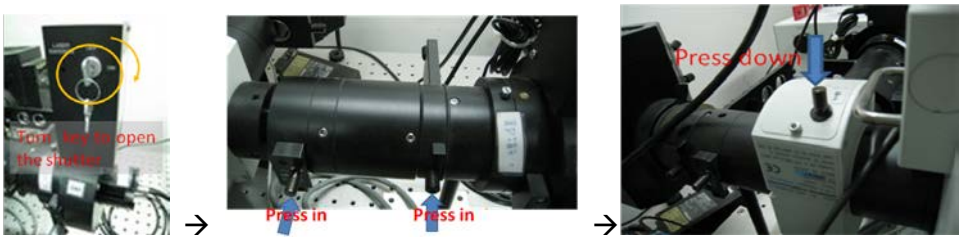


4. 488 nm (Blue)：Turn the key after 1 min. from switched on the power supply (the right).
5. 532 nm (Green)：Turn the key after 1min. from switched on the power supply(on the back)(the middle)
6. 638 nm (Red)：Switch on the power supply (on the back) after check both of the front switches up (the left).

7. Pull up the shutter of the laser that you need, and turn the knob clockwise to adjust the output power (≦20%).



8. Open the shutters in the optical path way as follows:



9. Switch off：
   (1) Return all the knobs to zero.

(2) Close all the shutters.

(3) **488 nm (Blue):** Turn the key first, then switch off the power supply after the fan was silence (required about 5~10 min.).

532 nm (Green): Turn the key, and wait for cooling down (about 1 min.), and then switch the power supply sequentially.

638 nm (Red): Switch the power supply off. (Due to the better cooling system)

10. Write the use record.

NOTE: **DO NOT** look straight ahead of the exit of the optical fiber, and avoid hits, breaks and snap, etc... of the fiber and joints.

I. TIRF

1. Check the book of user record.
2. Turn on the Pc1.
3. Turn on the XYZ-stage-controller, and check that if the Z-position-controlled is switched to "disengaged". If it's not at the status of "disengaged", switch to "disengaged".
4. The X- or Y-axis of the sample is controlled by the XYZ-stage-controller. Only, the Z-position is controlled by the "focusing knobs" on the IX71 microscopy.

   NOTE: If there is noise from the knob or it moves automatically, please stop operating it and contact the super-user immediately.

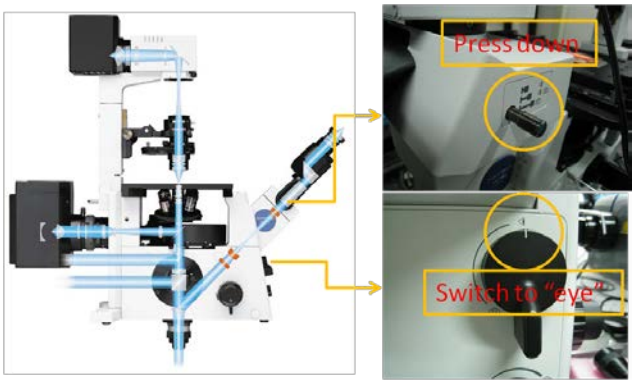
5. Switch on the Laser

   SEE: **H. Laser**

6. Lower the stage for protecting it from collision between the sample and the objective and install the 60x special objective lens (for TIRF) which is pleased in the dry cabinet (clean it before use), then turn the ring (cover-slip thickness) to 0.17 (mm).
7. Choose the filter cube (No.4→ for 488 nm laser, No.5→ for 532nm laser, No.6→ for 638nm laser) and take off the excitation filter.

   SEE ALSO: **B. 301_R Optical System – 4. Filter Cube**

8. Eyepiece switch: Upper one in the figure below: full press in the bar, lower one in the figure below: switch to the "eye icon".

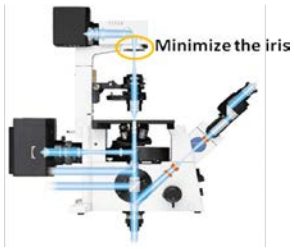


9. Press the bar down to let the light from Laser can pass.

10. Open the laser shutter and adjust the intensity.

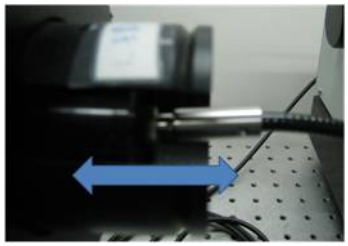
11. Turn the key to open the shutter of TIRF.

12. Minimize the iris.

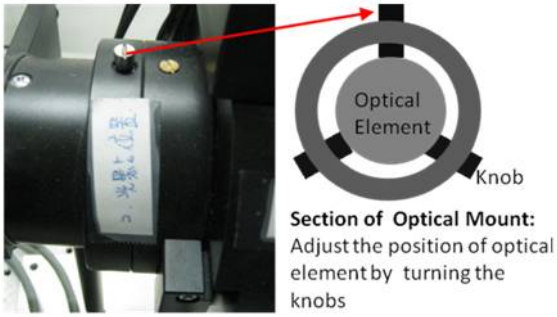


13. Zero the TIRF knob (as shown below).

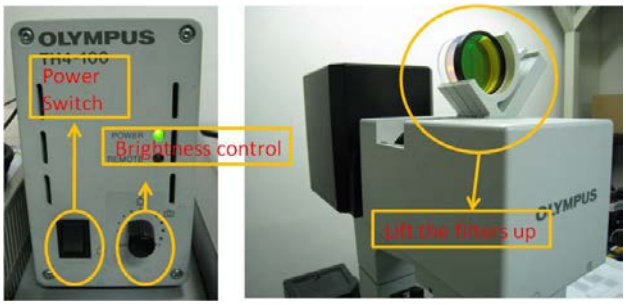
14. Move the fiber forward or backward to minimize the spot size.



15. Move the laser spot to the center on the iris by adjusting the optical mount.



16. Close the shutter(s) of laser(s).
17. Switch the halogen lamp on, if it's needed. Make suited brightness for the observation and lift the filters up.



18. Drop the oil on the objective (about 1~2 drops) and place the sample (upside down, as shown below).

19. Raise the objective lens holder slowly till the objective lens touch the sample, and find the focus plane by turning the focus knob.
20. Turn off the halogen lamp and then open the shutter of laser.
21. Turn the TIRF knob to make the laser spot disappeared.
22. Turn the specimen focus knob to find the focus plane with brightest fluorescence.
23. Use EM CCD or Pixel-Fly CCD to record. In this function, there're two CCDs can use, just choose the one which is suited to your experiment.

    SEE: **E. EM CCD** or **F. Pixel-Fly CCD.**

24. Download the data by DVD or VCD only. NOT USB.

25. Turn off Laser(s)

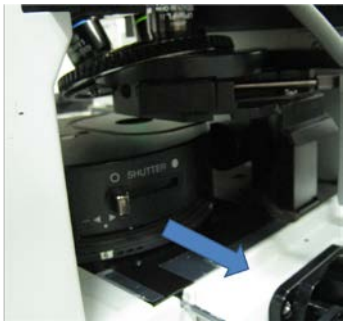
    SEE: **H. Laser.**

26. Turn off the PC1 after data-download.
27. Switch off the XYZ-stage controller.
28. Switch all lamps off.

29. Lower the stage and take off the objective lens.
30. Use the cleaning paper and hemostat to clean the oil objective lens with the MeOH or EtOH(as shown follows), and then put it into the dry cabinet.

31. Close the filters.

32. Load the excitation filter back and replace the cube on the holder of microscopy.

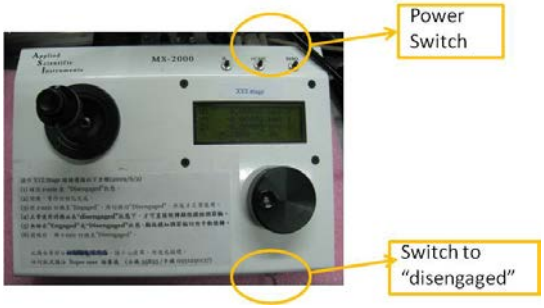


33. Clean the table.
34. Write the user record.

J. Confocal Scanning System (Based usage, NO auto-z-axial scanning)

1. Check the user record.
2. Turn on PC1 & PC2, and then use the Remote Desktop Control (RDC) to control the PC2 by the PC1 (click “NE301.RDP”on the desktop of PC1, P.W.:35807) (if it can’t be connected, please try it again after 5-10min)

3. Turn on the XYZ-stage-controller, and check that if the Z-position-controlled is switched to “disengaged”. If it’s not at the status of “disengaged”, switch to “disengaged”



4. The X- or Y-axis of the sample is controlled by the XYZ-stage-controller. Only, the Z-position is controlled by the “focusing knobs” on the IX71 microscopy.

   NOTE: If there is noise from the knob or it moves automatically, please stop operating it and contact the super-user immediately.

5. Lower down the stage for protecting it from collision between the objectives and the sample and then install an appropriate objective (In this function, 60x/NA. 1.25, 100x/NA. 0.6-1.3 or 100x/NA. 1.3 oil objective lens can be used, and all of them always be put in the dry cabinet for storage).

   SEE ALSO: **B. 301_R Optical System – 3. Objective lenses**

6. Place the sample (upside down, as shown below).

7. Find the focal plane of sample by bright field、fluorescence、phase contrast or dark field

microscopy.

SEE: **C. Based Use of Microscopy.....** or **D. Phase contrast and Dark Field Microscopy.**

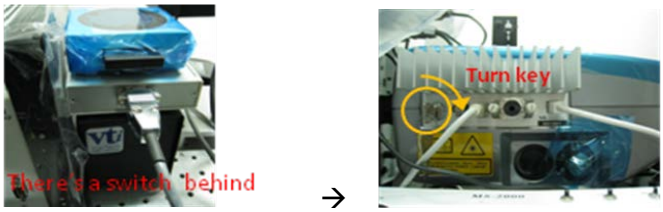
8. Close all the shutters and intensity knobs along the optical pathway of laser, then turn on the laser that you need.

   SEE: **H. Laser.**

9. Pull out the laser fiber (left figure) and load it into the scanning system (right figure).

   NOTE: **DO NOT** look straight ahead of the exit of the optical fiber, and avoid hits, breaks and snap, etc... of the fiber and joints.

10. Switch on the controller of the scanning system (behind the microscopy), then turn the key to open the shutter.

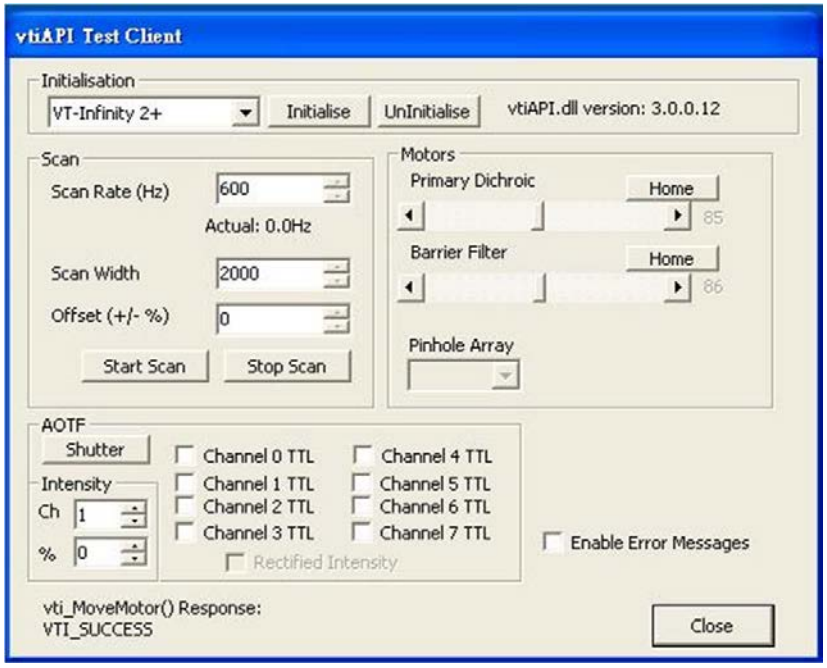


11. Click "VTI confocal" on the desktop, PC2, to control the scanning system.
12. **Setup the parameters of "VTI Confocal"** (see also the figure below)
    (1) Initialisation: choose "VT-Infinity V2+"→and then click "initialize".
    (2) Scan Rate(Hz): 600 Hz.
    (3) Scan Width: 2000 (unit: μm).
    (4) Adjust the filters inside scanning system. As shown in the table below.

	Dichronic	**BF**
488 nm	**130**	**122**
532 nm	**85**	**86**
638 nm	**40**	**50**

NOTE: It must be **very accurate** in filter-adjusting. **NO error can be allowed.**

(5) Click “Start Scan”.



13. Switch on the iCCD (**DO NOT** use the EM CCD neither do Pixel-fly CCD at the same time).

14. Click “CamWare” on the desktop, PC1.

15. Click for a new window of black/ white vision **(iCCD can take black/white image only)**

16. Click to setup the Parameters of “Camware”

(1) Mode → Single Trigger。

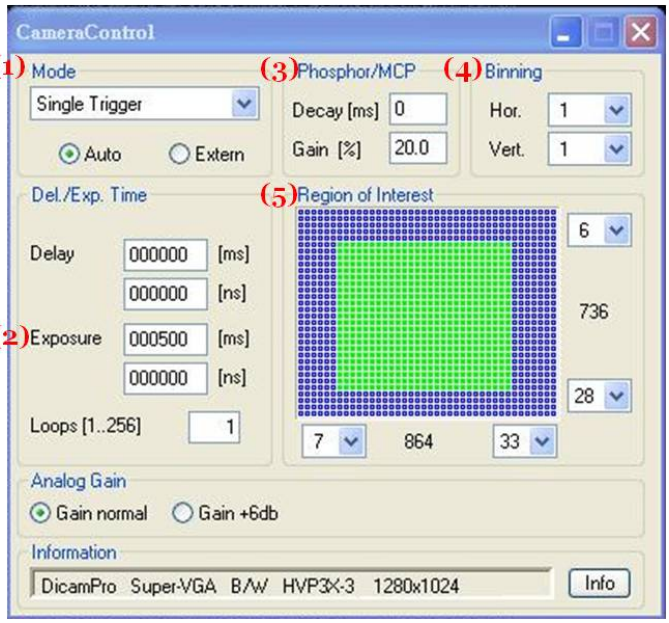
(2) Del./Exp. Time → Exposure: 10 ms 。

(3) Gain [%]: 0。

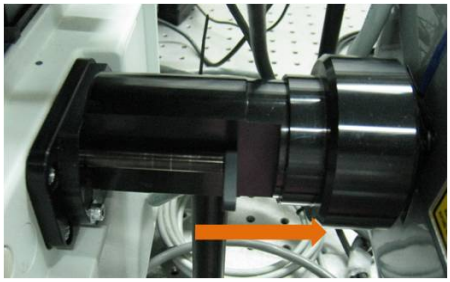
<u>NOTE:</u> **The exposure time always begin 10 msec., and the increase can’t over 5 times of the last value, and Gain value can only increasing 10% from 0% every time, and the maximal value is 50%.**

(4) Bining: horiz.→1, vert.→1.

(5) Region of Interest:horiz.→8-32, vert.→8-27.



17. Pull out the bar to switch to the confocal system. (as shown below)



18. Open the shutter of laser, and adjust the output power (begin from 1.5%).
19. Choose the cube No.1 (50/50 BS)

    SEE ALSO: **B. 301_R Optical System – 4. Filter Cube**

20. **Double check the filters inside the scanning system in step 12.**
21. The usage of "CamWare" of iCCD is similar as EM CCD and Pixel-fly.

    SEE ALSO: **E. EM CCD (Electron-Multiplying……)** or **F. Pixel-fly.**

22. Turn off laser(s).

    SEE: **H. Laser.**

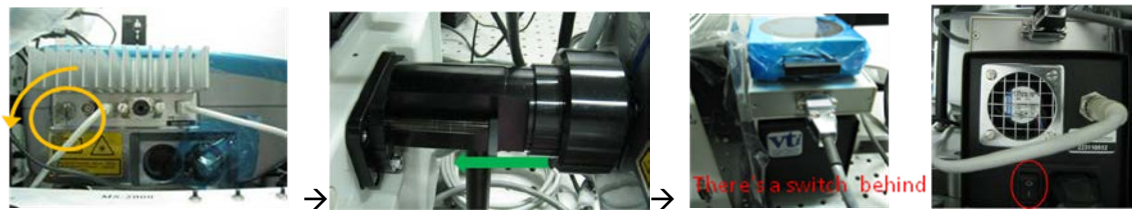
23. **Download the data by DVD or VCD only. USB is NOT allowed.**
24. Reset the parameters of "VTI confocal". PC2.
    (1) Click "Stop Scan".
    (2) click "Uninitialize"
    (3) Click "Close" to close "VTI confocal".
25. Turn off the PC2(Move the mouse to the toolbar and click the right button of mouse →工作管理員(K) →關機(U) →關機(U) →確定)
26. Reset the parameters of "CamWare". PC1.

(1) Mode → Single Trigger.

(2) Del./Exp. Time → Exposure: 10 ms .

(3) Gain [%]: 0.

27. Close “CamWare”.
28. Switch off iCCD
29. Turn off PC1.
30. Switch off the XYZ-stage controller.
31. Load the fiber back into TIRF and tape the jack of scanning system with blue tape.
32. Turn the key(anti-clockwise) and press in the bar of confocal system, and then switch off the controller of scanning system. (As shown below)



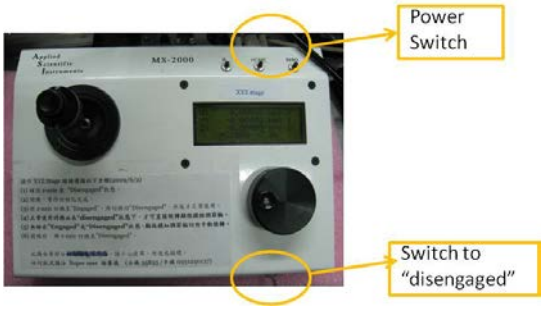
33. Lower the stage and take off the objective lens.
34. Use the cleaning paper and hemostat to clean the oil objective lens with the MeOH or EtOH(as shown follows), and then put it into the dry cabinet.

35. Clean the table.
36. Write the user record.

K. Confocal Scanning System (Auto-z-axial scanning)

1. Check the user record.
2. Turn on PC1 & PC2. And use the Remote Desktop Connection(RDC) to control PC2, password is ”35807”

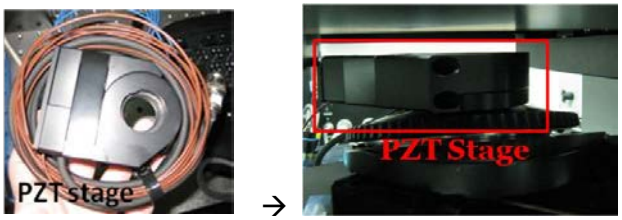
3. Turn on the XYZ-stage-controller, and check that if the Z-position-controlled is switched to “disengaged”. If it’s not at the status of “disengaged”, switch to “disengaged”



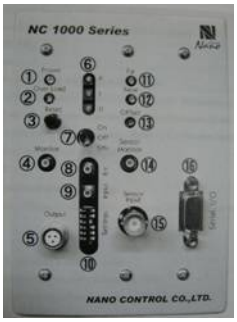
4. The X- or Y-axis of the sample is controlled by the XYZ-stage-controller. Only, the Z-position is controlled by the “focusing knobs” on the IX71 microscopy.

   NOTE: If there is noise from the knob or it moves automatically, please stop operating it and contact the super-user immediately.

5. Load the PZT stage on the objective lens holder.(As shown below)



6. Turn on the amplifier (NC 1000 Series).(The switch is on the backside)



7. Link up the two wires of PZT stage with DAQ box on the PC1 (AO 0 and AI 0).(As shown below)

8. Lower down the stage and then install an appropriate objective (In this function, 60x/NA. 1.25, 100x/NA. 0.6-1.3 or 100x/NA. 1.3 oil objective lens can be used, and all of them always be put in the dry cabinet for storage).

   SEE ALSO: **B. 301_R Optical System – 3. Objective lenses**

9. Place the sample (upside down, as shown below).

10. Find the focal plane of sample by bright field、fluorescence、phase contrast or dark field microscopy.

    SEE: **C. Based Use of Microscopy.....** or **D. Phase contrast and Dark Field Microscopy.**

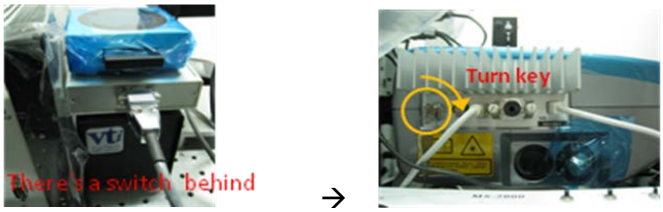
11. Close all the shutters and intensity knobs along the optical pathway of laser, then turn on the laser that you need.

    SEE: **H. Laser.**

12. Pull out the laser fiber (left figure) and load it into the scanning system (right figure).

NOTE: **DO NOT** look straight ahead of the exit of the optical fiber, and avoid hits, breaks and snap, etc... of the fiber and joints.

13. Switch on the controller of the scanning system (behind the microscopy), then turn the key to open the shutter.

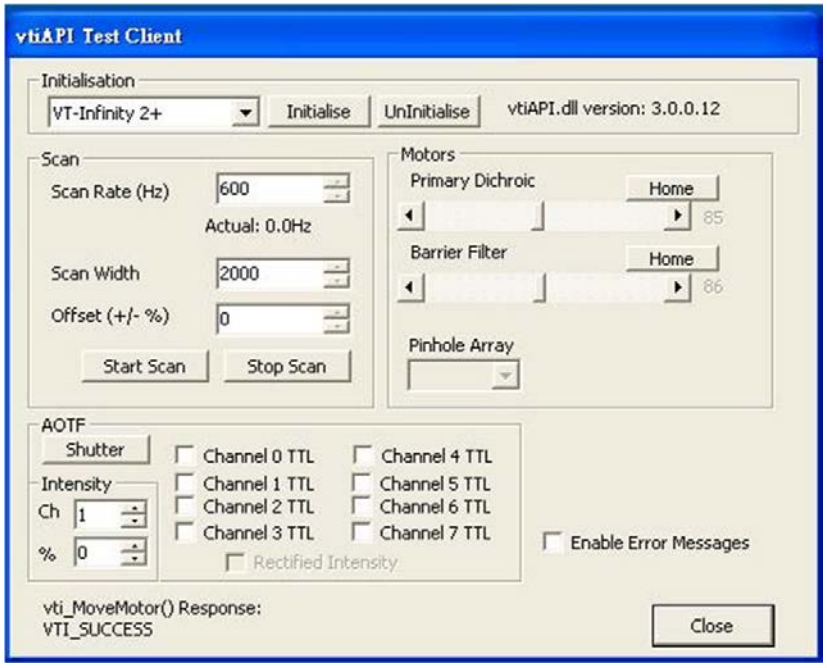


14. Click "VTI confocal" on the desktop, PC2, to control the scanning system.
15. **Setup the parameters of "VTI Confocal"** (see also the figure below)
    (1) Initialisation: choose "VT-Infinity V2+"→and then click "initialize".
    (2) Scan Rate(Hz): 600 Hz.
    (3) Scan Width: 2000 (unit: μm).
    (4) Adjust the filters inside scanning system. As shown in the table below.

	Dichronic	**BF**
488 nm	**130**	**122**
532 nm	**85**	**86**
638 nm	**40**	**50**

NOTE: It must be **very accurate** in filter-adjusting. **NO error can be allowed.**

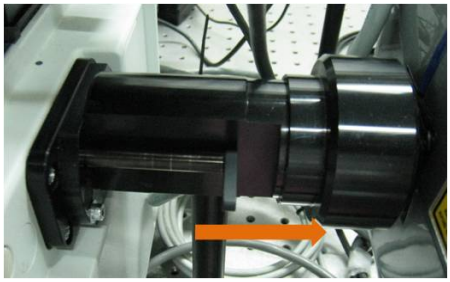
    (5) Click "Start Scan".



16. Switch on the iCCD (**DO NOT** use the EM CCD neither do Pixel-fly CCD at the same time).

17. Turn on the DAQ box(the switch is behind the DAQ box)

18. Pull out the bar to switch to the confocal system. (as shown below)



19. Open the shutter of laser, and adjust the output power (begin from 1.5%).
20. Choose the cube No.1 (50/50 BS)

SEE ALSO: **B. 301_R Optical System – 4. Filter Cube**

21. **Double check the filters inside the scanning system in step 12.**

22. Click “LabView” on desktop, PC1, and click to run the program.

23. Click

.

24. Parameters setup:

(1) Exposure time: 300 msec.. **The exposure time always begin 300 msec..**

(2) Gain = 0%. **Gain value can only increasing 10% every time, the maximal value is 50%.**

(3) Scanning range: horiz.→8~32, vert.→8~27.

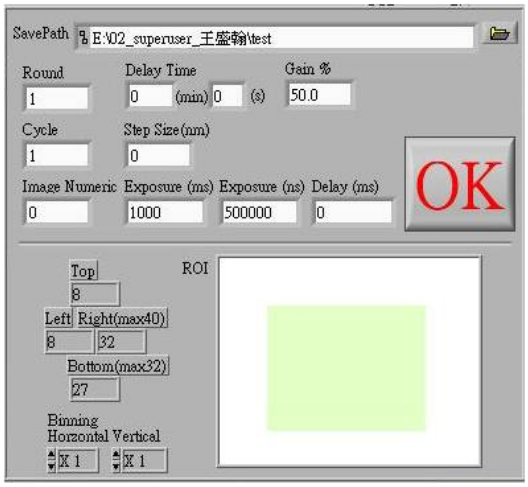
(4) Binning: horiz.→1, vert.→1.

(5) Adjust the contract bar to the suited value (about 500~1000, depend on the user) on the left of the window.

(6) Click “OK”.

25. Click

and then set up the Round/ Cycle/ Numeric Number、delay time (>10 sec.)、Step size (50~5000 nm) and folder (the data will be save in, after choose the position of the folder, click “current folder”) →click “OK”.



26. After system finish the work, please click “確定”,and then it will auto export as 8 bit and 16 bit *.tif* and the parameters into the folder that you choose.

27. Close “Labview”.

28. Turn off laser(s).

SEE: **H. Laser.**

29. Download the data by DVD or VCD only. NOT USB.

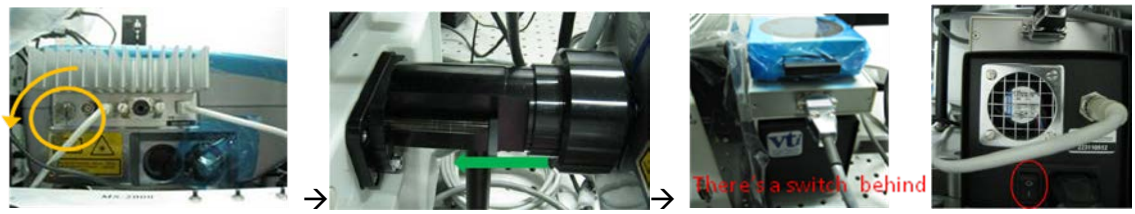
30. Reset the parameters of “VTI confocal”, PC2.

(1) Click “Stop Scan”.

(2) VT-Infinity V2+→uninitialize

(3) Close “VTI confocal”.

31. Turn off the PC2(Move the mouse to the toolbar and click the right button of mouse →工作管理員(K) →關機(U) →關機(U) →確定)
32. Switch off iCCD and amplifier.
33. Take off the wires of PZT stage from DAQ box and turn off DAQ box.
34. Turn off PC1.
35. Switch off the XYZ-stage controller.
36. Load the fiber back into TIRF and tape the jack of scanning system with blue tape.
37. Turn the key(anti-clockwise) and press in the bar of confocal system, and then switch off the controller of scanning system. (As shown below)



38. Lower the stage and take off the PZT stage and objective lens.
39. Use the cleaning paper and hemostat to clean the oil objective lens with the MeOH or EtOH(as shown follows), and then put it into the dry cabinet.

40. Clean the table.
41. Write the user record.

Appendix User List

權限 level	一般時段可預約上限	姓名 name	合格項目						訓練其他使用者(填日期與被訓練者)
			明視野暨螢光顯微鏡 Bright Field and Fluorescence	暗視野 Dark Field	Laser	TIRF	Spectrometer	Confocal system	
Chief Superuser	12 hr.	王盛翰	V	V	V	V	V	V(auto Z)	NONE
			分機:02-27823212#893 手機:0911205217 e-mail: hedgren-ix@hotmail.com						
Superuser	12 hr.	謝馨儀	V	V	V	V	V	V(auto Z)	NONE
			分機:35833 手機:0931250137 e-mail: d9735803@oz.nthu.edu.tw						
Superuser	12 hr.	王政輝						V(auto Z)	NONE
			分機:35807 手機:0986335855 e-mail: abcd5855tw@yahoo.com.tw						
Superuser									
			分機: 手機: e-mail:						
user	6 hr.	蕭建隆	V	V	V			V(auto Z)	
			分機:02-27898000#53 手機:0911314438 e-mail: jlxiao@gate.sinica.edu.tw						
user	8 hr.	陳宗儒	V		V	V		V	
			分機:34306 手機:0922760091 e-mail: peterchen810@gmail.com						
user	6 hr.	Tuhin	V		V	V		V	
			分機:34306 手機:0975314941 e-mail: santra.tuhin@gmail.com						
user	6 hr.	黃蘊慈	V		V	V		V(auto Z)	
			分機:42685 手機:0921213943 e-mail: d924216@oz.nthu.edu.tw						
user	6 hr.	莊娟涓	V	V	V	V		V(auto Z)	
			分機:35833 手機: 0933029011 e-mail: aler_injuan@hotmail.com						
user	6 hr.	Judy					V		
			分機: 34306 手機:0975144901 e-mail: jmobliosca@yahoo.com						
user	6 hr.	Hang	V	V	V	V	V		
			分機: 34298 手機:0918236454 e-mail: d947129@oz.nthu.edu.tw						
user									
			分機: 手機: e-mail:						
user									
			分機: 手機: e-mail:						